

DOI: 10.19615/j.cnki.1000-3118.190425

Metatarsal II osteohistology of *Xixianykus zhangi* (Theropoda: Alvarezsauria) and its implications for the development of the arctometatarsalian pes

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Abstract The late-branching alvarezsaurian *Xixianykus zhangi* is among the smallest known non-avialan theropods. With great similarity to its close relatives, it is highly cursorial as indicated by proportionally long lower segments of the hindlimbs and the presence of an arctometatarsalian pes—a highly modified structure that has been suggested to improve cursorial capability in theropods. Here we describe the osteohistology of the metatarsal II of the holotype of *X. zhangi* (XMDFEC V 0011). Two rarely reported histological features, radial vascular canals and Sharpey's fibers, are presented in this study. We suggest that both features are related to the development of the arctometatarsalian pes; however, further investigations of metatarsal osteohistology in theropods are required for the validation of our interpretation.

Key words osteohistology, alvarezsaurian, arctometatarsalian, radial vascular canals, Sharpey's fibers

Citation Qin Z C, Zhao Q, Xu X, in press. Metatarsal II osteohistology of *Xixianykus zhangi* (Theropoda: Alvarezsauria) and its implications for the development of the arctometatarsalian pes. *Vertebrata PalAsiatica*. DOI: 10.19615/j.cnki.1000-3118.190425

1 Introduction

Alvarezsauria is an early-branching subgroup of maniraptoran theropods with a nearly global geographical distribution (Bonaparte, 1991; Perle et al., 1993; Karhu and Rautian, 1996; Novas, 1997; Chiappe et al., 1998; Hutchinson and Chiappe, 1998; Naish and Dyke, 2004; Martinelli and Vera, 2007; Alifanov and Barsbold, 2009; Longrich and Currie, 2009; Xu et al., 2010, 2011; Agnolin et al., 2012; Averianov and Sues, 2017). The late-branching members of this group are highly cursorial animals, as indicated by two features—extremely long lower segments of the hindlimbs and arctometatarsalian pes. The latter is characterized by a laterally compressed metatarsal III that contributes to a highly compacted metatarsus and has been

国家自然科学基金(批准号：41688103, 41120124002)资助。

收稿日期：2019-03-26

suggested to improve cursorial capabilities (Holtz, 1995; Snively et al., 2004). *Xixianykus zhangi* is a late-branching alvarezsaurian theropod from the Upper Cretaceous Majiacun Formation of Xixia, Henan Province, China (Xu et al., 2010) and similar to other late-branching alvarezsaurians, it has an extremely modified arctometatarsalian pes that is featured by a significantly short metatarsal III producing an even more compacted metatarsus (Xu et al., 2010). Here, we study the pedal osteohistology of the holotype of *X. zhangi* (XMDFEC V 0011) to investigate the development of the highly modified pes.

2 Material and method

We sampled the right metatarsal II of the holotype of *Xixianykus zhangi*, XMDFEC V 0011 (Xixia Museum of Dinosaur Fossil Eggs of China). In this study, two ground sections were made based on the distal end of the right metatarsal II (Fig. 1).

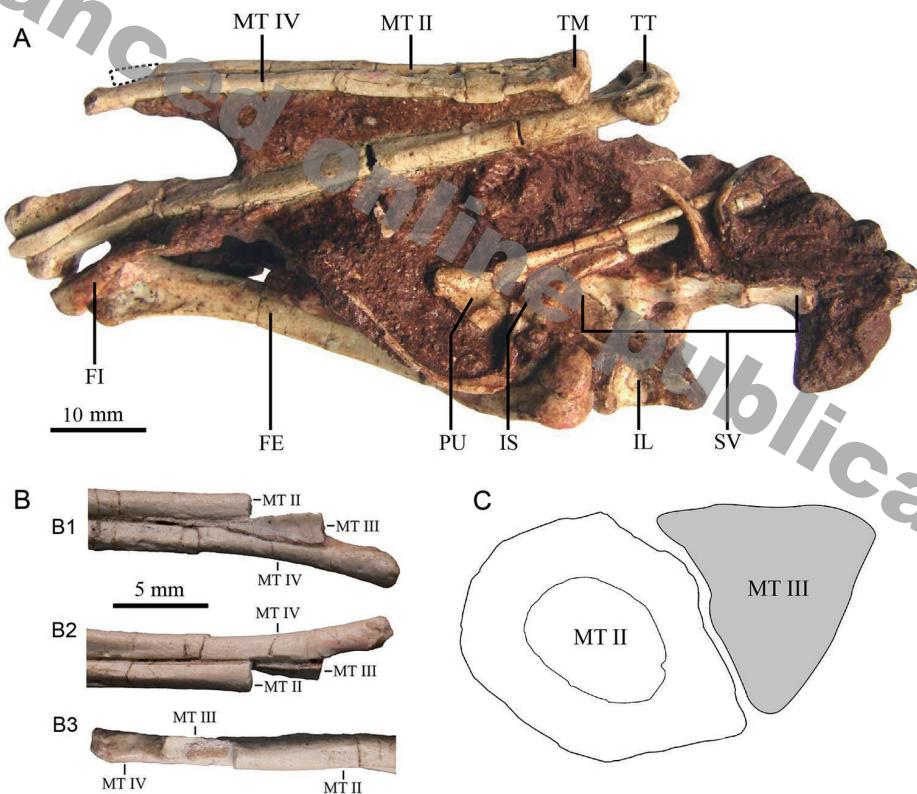


Fig. 1 The holotype of *Xixianykus zhangi* (XMDFEC V 0011) and the sketch diagram for where the ground sections sliced

A. the holotype of *X. zhangi* (XMDFEC V 0011) in ventral view (a dotted line indicates the sampled position); B. the dorsal (B1), ventral (B2) and medial (B3) views of its distal portion of right tarsometatarsus of *X. zhangi*; C. the sketch diagram showing the spatial relation between the metatarsals II and III on the thin section (the metatarsal II colored in white is where we histologically sectioned). C is no scaled.

Abbreviations: FE. femur; FI. flange; IL. ilium; IS. ischium; MT II. metatarsal II; MT III. metatarsal III; MT IV. metatarsal IV; PU. pubic; SV. sacral vertebrae; TM. tarsometatarsus; TT. tibiotarsus

Histological sections used in this study were prepared using standard techniques (Padian and Lamm, 2013). The distal end of the preserved portion of the right metatarsal II was embedded in resin, and the diaphyseal transverse sections were made using a diamond circular saw fitted with a diamond-tipped wafering blade. One surface of each ground section was polished with a wheel grinder/polisher (EXAKT400CS) by rough emery paper (500 grit), and then grounded using emery paper with finer grit (4000 grit) to produce a smooth texture ideal for the adhesion to a glass slide. The ground sections were then cut to a thickness of about 250 μm with a diamond circular saw before being ground down further to the desired final thickness of 80 μm (ground section I) and 50 μm (ground section II), leaving the exposed surface of the section smooth. The ground section I is more distally located than the ground section II, the distance between them is about 1–2 mm. Each slide was then cleaned in a water-filled ultrasonic cleaner to remove microscopic grit and finally capped with a glass coverslip. The completed two thin sections were studied in normal and polarized light, observed with Zeiss Axio Imager A2 microscope, and photographed with ZEN software from Zeiss.

3 Histological description

Both ground sections display compact primary cortices with sparse primary vascular canals (Figs. 2A, 3A). The medullary cavity is elliptical and filled with crystals (Fig. 3A). Two lines of arrested growth (LAGs) are identified (Fig. 2) and have been interpreted as a one-year growth record (Chinsamy, 2005). Though fibrolamellar bone occupies most of the cross section, the endosteal bone forms an uninterrupted band of lamellar bone laying alongside what used to be the endosteum. Longitudinal primary vascular canals and osteocyte lacunae dominate the bone cortex (Fig. 2B).

Ground section I, which has a thickness of 80 μm , displays radially arranged vascular canals in most of the cortex except for the ventral half of the lateral portion, and for the endosteal bone (Fig. 2A). These radial canals cross the growth marks without any morphological changes, indicating a multiyear forming structure (Fig. 2C). These radial canals are about 100–300 μm apart (Fig. 2C). Most of those in the lateral portion of the ground section are approximately 200 μm long, and those in the other portions of the ground section are variable in length and in general much shorter. Most radial canals are oriented perpendicularly to sub-perpendicularly to the periosteum. They reach close to the periosteum on the lateral and medial sides but are much deeper within the cortex and far from the periosteum on the dorsal side.

In ground section II, which has a thickness of 50 μm , Sharpey's fibers can be seen on the lateral side when observed under polarized light (Francillon et al., 1990; Holger and Sander, 2013). Under polarized light equipped with a quartz interferometer plate, Sharpey's fibers emerge as a blue-colored filamentous structure (Fig. 3A). Since the ground section I is thicker, Sharpey's fibers are hardly observed.

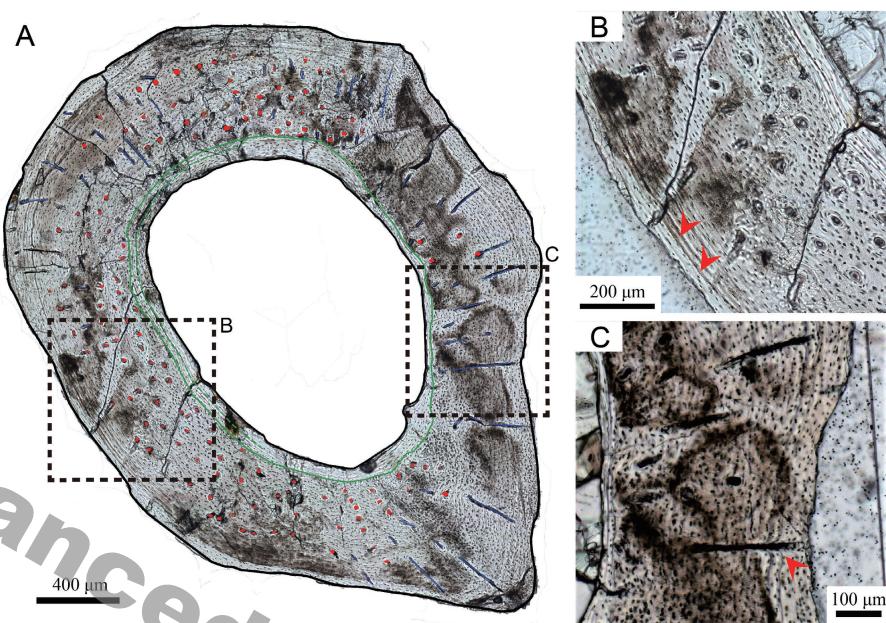


Fig. 2 The micrograph of the ground section I of metatarsal II of *Xixianykus zhangi* (XMDFEC V 0011) seen under normal light

A. the whole section under the normal light (red dots, primary canals; blue lines, radial canals; green line, endosteal bone boundary); B. the medial portion of the slice is dominated by longitudinal canals (two LAGs are indicated by two red arrows); C. a radial canal lies across the two LAGs (indicated by a red arrow)

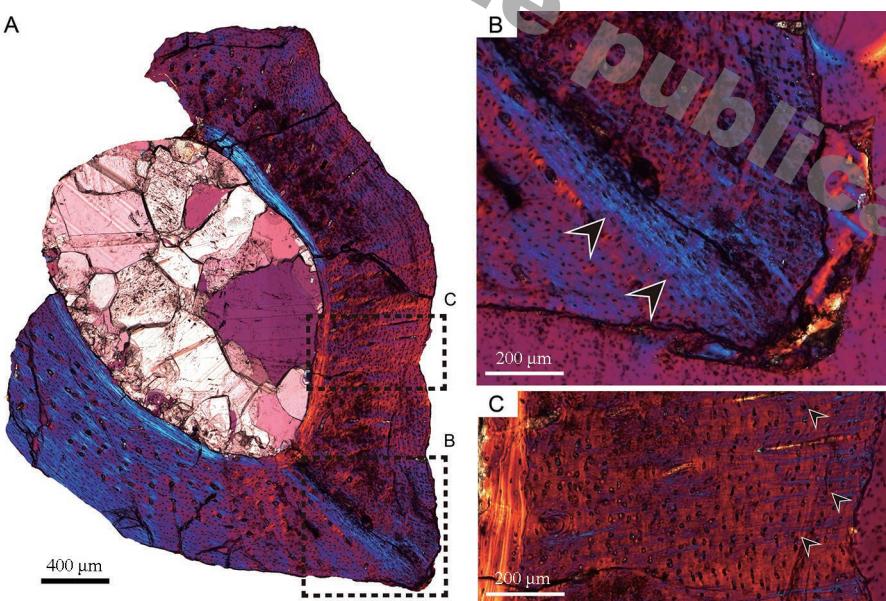


Fig.3 Microphotograph of the ground section II of metatarsal II of *Xixianykus zhangi* (XMDFEC V 0011) under polarized light

A. whole cross section observed under polarized light;
B. Sharpey's fibers can be seen in the lateroventral corner of metatarsal II (indicated by the black arrows);
C. sparsely distributed Sharpey's fibers on the lateral side of metatarsal II (indicated by the small black arrows)

Near the lateroventral portion, what appears to be a bundle of radial Sharpey's fibers can be seen. This bundle is obliquely oriented to the ventral periosteal surface (with an angle of approximately 40°, Fig. 3B). More dorsally in the lateral portion of the ground section are more sparsely distributed, much shorter Sharpey's fibers. These fibers are parallel to each other and to nearby radial canals, and they are nearly perpendicular to the periosteal surface (Fig. 3C).

4 Discussion

The main histological features observed in the metatarsal II of *Xixianykus zhangi*, including proportionally large medullary cavity, fibrolamellar-bone-dominated cortex, are similar to those in the long bones of other small-sized theropods, such as the *Shuvuuia* and *Archaeopteryx* (Erickson et al., 2001, 2009). For example, the medullary cavity is proportionally large, the cortex is mainly composed of fibrolamellar bone, longitudinal vascular canals dominate the cortex and are relatively sparse in distribution (Erickson et al., 2001, 2009).

Two histological features, the radial vascular canals and the Sharpey's fibers, are particularly interesting. Sharpey's fibers were first reported in alveolar tooth sockets of mammals as part of the periodontal ligament that can be traced through dental tissues (Sharpey, 1848). Sharpey's fibers also referred to connective tissue fibers that continuous with skeletal tissues and their enthesis. Sharpey's fibers also function as the binding of cranial bones and attaching cranial muscles to bone periosteum (Pritchard et al., 1956; Jones and Boyde, 1974; Nicaise et al., 1988; Hieronymus, 2006). Sharpey's fibers have been reported in extinct taxa, and have been suggested that they functioned as same as in extant animals (Chinsamy, 2005; Francillon et al., 1990; Holger and Sander, 2013). Among Mesozoic theropods, Sharpey's fibers have been reported in the tibia of the ceratosaurian *Masiakasaurus knopfleri* (Lee and O'Connor, 2013), the femur of an unnamed ceratosaurian (Canale, 2016), the hindlimb of the early-branching avialan *Confuciusornis* (Ricqlès et al., 2003), and the quill knobs of a few early-branching avialans (Wang, 2012). In the first three examples, Sharpey's fibers were probably used for muscle attachment whereas in the last case, they have been suggested to represent the insertion sites of feathers to bone (Wang, 2012). Sharpey's fibers were also suggested to be present in the metatarsals of some tyrannosaurids and in *Allosaurus* based on intermetatarsal rugose facets (Snively and Russell, 2003), though in the absence of any histological evidence.

Our observation represents the first histological evidence for the presence of Sharpey's fibers in the pes of any Mesozoic theropod. Sharpey's fibers in XMDFEC V 0011 include two different types: 1) obliquely oriented, densely-bunched ones in the lateroventral corner of the cross section of metatarsal II; and 2) parallelly arranged and sparsely distributed ones in the lateral portion of the cross section of metatarsal II. In living mammals, the Sharpey's fibers

found at the junction of cranial bones are sparser than those found at muscle attachment (Jones and Boyde, 1974). The densely-packed Sharpey's fibers seen in the lateroventral corner of the cross section of metatarsal II of XMDFEC V 0011 were more likely to be used for a tendinous attachment, whereas the parallelly arranged and sparsely distributed ones were most likely part of a ligamentous sheath surrounding and binding the metatarsal bones together, as in living mammals where Sharpey's fibers are used to bind cranial bones in a firm yet slightly movable manner (Pritchard et al., 1956).

In living birds, radial canals represent extremely rapid growth (Margerie et al., 2004; Montes et al., 2010), but they are also known in two other conditions such as in pathological bones and in bones associated with substantial shape changes (Erickson and Tumanova, 2000). Radial vascular canals were not reported in other small theropods such as *Mahakala* or *Archaeopteryx* (Erickson et al., 2009), nor other alvarezsaurians including *Shuvuuia deserti*, *Albinonykus Baatar*, *Xiyunykus pengi* and *Bannykus wulatensis* (Erickson et al., 2001; Nesbitt et al., 2011; Xu et al., 2018). They are also unknown in giant theropods (Erickson et al., 2004, Horner and Padian, 2004) and were not found even in sauropods (Curry, 1999; Klein and Sander, 2008), presumably because these gigantic dinosaurs had lower maximum growth rates and bone deposition rates than those of living birds (Margerie et al., 2004; Montes et al., 2010; Erickson, 2014).

However, radial vascular canals are known to be present in the ceratopsians *Psittacosaurus mongoliensis* (Erickson and Tumanova, 2000) and *P. lujiatunensis* (Zhao et al., 2013), and the hadrosaurian *Maiasaura* (Cubo et al., 2015). In these cases, the radial vascular canals were suggested to be related to substantial shape changes as a result of loading change (Erickson and Tumanova, 2000; Zhao et al., 2013; Cubo et al., 2015). Radial vascular canals were also reported in the early-branching sauropodomorpha *Plateosaurus engelhardti* but the interpretation of these structures was ambiguous (Klein, 2004).

Our report of the presence of radial vascular canals in XMDFEC V 0011 is the first one in any theropod. They differ from the radial vascular canals that were identified as resulting from a pathology, like those found in a giant dinosaur (identified as a possible ornithopod or sauropod) in the following criteria (Chinsamy and Deratzian, 2009): radial canals are 100–300 μm apart from each other rather than densely bunched; radial canals are oriented perpendicularly to the periosteum rather than parallelly; radial canals extend from the endosteum to the periosteum instead of limited to near the periosteum. Radial vascular canals in XMDFEC V 0011 are probably related to substantial shape changes, as suggested for other dinosaurs (Erickson and Tumanova, 2000; Zhao et al., 2013; Cubo et al., 2015). More specifically, the lateroventral portion of metatarsal II expands significantly laterally to buttress the ventrally thin metatarsal III to form the arctometatarsal condition.

The presence of Sharpey's fibers and radial vascular canals in metatarsal II of *Xixianykus* shed new light on how the arctometatarsal condition forms. The former feature explains how the metatarsals connect in a firm but movable manner, which is important for cursorial capability; the latter feature explains the substantial shape change, seen at the histological

level, that occurs during the development of the arctometatarsal condition. However, more histological investigations of theropod metatarsals, especially in the arctometatarsalian pes, are needed to confirm this interpretation.

Acknowledgements We thank Ding Xiaoqing for preparing specimens, Zhang Shukang for guidance of manufacturing histological slices, and Chen Guanhua for photographs. We thank P. Martin Sander from University of Bonn for the discussion. We thank Alida Bailleul for the discussion and her comments on the manuscript. This study was supported by the National Natural Science Foundation of China (41688103 and 41120124002).

张氏西峡爪龙(兽脚类: 阿尔瓦雷斯龙类)第二跖骨的骨组织学 及其对“窄足型”足部结构发育的启示

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摘要: 阿尔瓦雷斯龙类的一晚期演化支——张氏西峡爪龙(*Xixianykus zhangi*), 是体型最小的非鸟兽脚类恐龙之一。与其他阿尔瓦雷斯龙类类似, 其胫跗骨和足部相对较长, 并具有善于奔跑的兽脚类恐龙中常见的窄足型足部, 指示其可能非常善于奔跑。对张氏西峡爪龙的第二跖骨进行了骨组织学研究, 发现了两种罕见的骨组织学结构: 放射状骨脉管和沙比纤维。认为这两种结构与其具有的窄足型足部有关, 然而还需要对更多的兽脚类跖骨进行骨组织学研究来验证这一结论。

关键词: 骨组织学, 阿尔瓦雷斯龙类, 窄足型足部, 放射状骨脉管, 沙比纤维

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